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Phytochemical Profiling, Heavy Metals Composition, *In Silico* Aphrodisiac Potential, and ADMET Study of *Gardenia erubescens*

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Abstract: This study aimed to explore the phytochemical profile, heavy metal composition, *in silico* aphrodisiac potential, and ADMET study of *Gardenia erubescens* due to its folkloric acclaimed aphrodisiac use. The phytochemicals were quantified gravimetrically while the identification of bioactive compounds was carried out using a combined Gas spectrophotometer-mass spectrophotometer (GC-MS). Heavy metals were quantified using an atomic absorption spectrophotometer while the aphrodisiac and ADMET studies were *in silico*. The result showed the presence of alkaloids (22.33% ±1.45), saponins (20.17% ±1.88), glycosides (0.55% ±0.03), and flavonoids (32.67% ±1.45), with the absence of steroids and terpenoids. GC-MS analysis identified 25 compounds with linoleic acid having the highest peak area (28.01%) next to palmitic acid (14.08%). Chromium, Cadmium, and Lead were present in concentrations of 0.145 ±0.03, 0.001 ±0.00, and 0.065 ±0.03 ppm respectively. Ethyl D-glucopyranoside had the least BA (-8) and Ki (1.35 µM) docked with human arginase II while Tyrosinol had the least BA (-6.2) and Ki (28.21 µM) docked with phosphodiesterase 5 though both were higher than Sildenafil citrate. All the top docked compounds were predicted to be neither substrates nor inhibitors of P-glycoproteins and cytochrome P450 enzymes without CNS permeability and hepatotoxicity. Conclusively, the present study supports the folkloric aphrodisiac application of *Gardenia erubescens*, and the heavy metals level was below the acceptable regulatory level, thus, might be safe for occasional use. Additionally, the identified compounds might be considered a novel source of therapeutics against erectile dysfunction.

Introduction

Impotence otherwise termed erectile dysfunction (ED) is a recurrent and persistent inability to achieve and/or keep sufficient erection for satisfactory intercourse following sexual stimulation (1). Erection or tumescence is a state of engorgement characterized by a flow of blood induced by neurotransmitters released from the cavernous nerves during sexual stimulation, though it occurs spontaneously (1). Causes of ED are classified based on conditions associated with hypoactive and normoactive sexual activity with the former covering attraction toward partners, ailments (including hypogonadism and hyperprolactinemia), and psychogenic conditions (2) while the latter covers metabolic, vascular, neurological, and inflammatory ailments (1). For centuries, the use of pharmaceuticals and aphrodisiacs was employed for the management of ailments, however, the current approach includes improvement in lifestyle and the use of drugs, notably the phosphodiesterase inhibitor sildenafil (1). Other approaches include nutraceuticals and physical and surgical treatments. Sildenafil has been previously associated with visual impairment and hepatotoxicity, stomach upsets, headaches, and nosebleeds (3-5). Medicinal plants with aphrodisiac activities have emerged as alternatives to sildenafil attributed to their minimized side effects (6-9).

Medicinal plants are vital for both traditional and modern medicine, and pharmaceutical industries. In

traditional medicine, medicinal plants are utilized in herb forms prepared in different forms taken orally, topically, or through inhalation for the treatment of ailments, especially in rural areas where there is poor healthcare delivery (10, 11). The synergy and low side effects of medicinal plants make them desirable especially considering their affordability compared to synthetic medicines. In modern medicine, different medicinal plants were reported to possess pharmacological properties thus, finding their way for utilization against different conditions such as cancer, diabetes, and bacterial, fungi, and viral infections (12, 13). In the pharmaceutical industries, medicinal plants serve as a vital source of bioactive compounds used in the synthesis of novel therapeutics. Different plants were reported to be associated with aphrodisiac pharmacological properties including *Gardenia erubescens* (GE) (12, 14).

The therapeutic roles of medicinal plants are credited to their phytochemical components made up of different bioactive compounds working individually or synergistically to produce pharmacological effects (15). Phytochemicals are substances produced by plants to perform important functions other than nourishment such as protection against pathogens (16). GE is a popular plant which is called Gaude in Northern Nigeria. In traditional practice, the root of the plant is utilized as an aphrodisiac while the aerial parts are applied in the management of gonorrhea and insomnia by herbalists (17, 18). The plant was also reported to exert moderate antioxidant, anti-obesity, and anti-plasmodial activity (14, 19). The application of *in silico* studies including molecular docking, molecular dynamics, and ADMET significantly improves the drug discovery and development process paving the way for wet lab and reducing cost and time in identifying lead compounds from a library of compounds. Additionally, this aspect allows for the improvement of the pharmacological properties of the lead compounds. Thus, in our study, we conducted the phytochemical profiling and determined the heavy metals composition and *in silico* aphrodisiac potential of ethanol extract of GE seeing it reported aphrodisiac application in traditional ethnomedicine, thus leading to heavy metal poisoning.

Experimental Section

Plant Material

A stem bark sample of the GE was collected from Girei Local Government, Adamawa state, Nigeria. A voucher specimen (ASP/FT/111) was deposited after identification by a Forest Technologist from the Forestry Technology Department of Adamawa State Polytechnic, Yola, followed by shade-drying and grinding using a blender.

Extract Preparation

The sample was extracted by maceration of 400 g of bark powder of GE in 1.5 L of 90% (v/v) ethanol for 48 h, followed by filtration and concentration to dryness in a rotary evaporator (Buchi Rotavapor R-200) at 40 °C to yield the ethanol stem bark extract (ESBE) of GE (20).

Qualitative Phytochemical Analysis

Phytochemicals present in the ESBE of GE were identified using the method reported previously to detect alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids (20). The chemicals and reagents used in the present were of AnarlaR obtained from Xilong Scientific Co., Ltd. Guangdong, China.

Quantitative Phytochemical Analysis

The quantification of phytochemicals in ESBE of GE was carried out by methods reported previously as follows:

Total Alkaloids Content

Alkaloids were quantified by the gravimetric method (21). Briefly, 0.5 g extract was introduced into a conical flask and 10 mL of 20% aqueous ethanol was added. The sample was heated over a water bath for 1 h with continuous stirring at about 550 °C. The concentrate was transferred into a 250 mL separator funnel and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded. About 10 mL of n-butanol was then added followed by the addition of 2 mL of 5% aqueous NaCl. The remaining solution was heated over a water bath. After evaporation, the sample was dried in the oven to a constant weight and calculated using **Equation 1**.

Saponins Content

Quantification of saponins was done by the method previously described (22). Exactly 0.5 g extract was dispensed into a conical flask and 10 mL of 20% aqueous ethanol was added. The sample was heated over a water bath for 1 h with continuous stirring at about 55 °C. The concentrate was transferred into a 250 mL separating funnel and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded. Exactly 10 mL of n-butanol was then added followed by the addition of 2 mL of 5% aqueous NaCl. The remaining solution was heated over a water bath. After evaporation, the sample was dried in the oven to a constant weight and calculated using **Equation 1**.

$$Total\ metabolites\ (\%) = \frac{Weight_{residue}}{Weight_{sample}} \times 100\% \quad \textbf{Equation 1}$$

Total Glycosides Content

Glycosides were quantified as described previously (23). Exactly 0.5 g of the extract was dispensed into a 100 mL volumetric flask containing 10 mL of 70% of ethanol. It was boiled for 2 minutes in a water bath, filtered and the filtrate was diluted with 20 mL of distilled water. Afterwards, 2 mL of 10% lead acetate was added to this volumetric flask to precipitate the chlorophyll, tannins, and alkaloids, followed by filtration. The filtrate was transferred to a separating funnel containing 10 mL of chloroform. The funnel was shaken by inverting repeatedly. Two layers were formed, and the lower organic layer was collected (chloroform); dried, and weighed. The percentage of total glycosides contents was determined using **Equation 1**.

Flavonoid Content

Quantification of flavonoids was carried out according to a method described previously (21). Exactly 0.5 g of the extract was mixed with 10 ml of 80% aqueous methanol. The whole solution was filtered through Whatman filter paper. The filtrate was transferred to a pre-weighed crucible and evaporated into dryness over a water bath weighed, and calculated using **Equation 1**.

Gas Chromatography-mass Spectrometry Analysis

GC-MS analysis was carried out with a combination of a Gas chromatography-mass spectrophotometer (Agilent 19091-433HP, USA), fitted fused with a silica column while the settings and compound identification were as we previously described (24).

Determination of Heavy Metal Composition

A gram of the samples was burned to ash at 500 °C for 1 h, dissolved in 25 mL of 10% HCl, and made up to 100 mL (25). Chromium (Cr), cadmium (Cd), and lead (Pb) contents were quantified by the method previously described (25) using an Atomic Absorption Spectrophotometer (AAS) (Buck Scientific AAS210).

Molecular Docking and Molecular Dynamics Simulation

The compounds identified in ESBE of GM were initially screened applying the Lipinski's rule and Veber filters using the DruLiTo software (https://niper.gov.in/pi_dev_tools/DruLiToWeb) predicting 7 with drug-likeness properties out of the 25. The structures of the 7 compounds and sildenafil citrate (standard drug) were downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov) in SDF format and energy minimized with PyRx virtual screening Tool software (version 0.8). **Table 1** shows the list of compounds and sildenafil citrate inclusive of their PubChem ID. The docking targets including Human Arginase II (HMA2) and Phosphodiesterase 5 (PDE5) with PDB IDs of 1PQ3 and 5ZZ2 respectively were downloaded from the RSCB database (https://www.rcsb.org) and prepared by removing identical chains, water molecules, and heteroatoms using AutoDockTools version 1.5.7 (26). The docking pockets (coordinates) for HMA2 (X= 69.73, Y= 54.15, and Z= -4.94) and PDE5 (X= 32.49, Y= -31.77, and Z= -37.40) were identified by the Prankweb online server (https://prankweb.cz) (27). The docking was carried out using the Vina wizard of the PyRx software. The inhibition constant (Ki) was evaluated from the binding affinity (BA) by the equation; $Ki = \exp \Delta G/RT$ where T=298.15 K (temperature) and R=1.985 x 10^{-3} $kcal^{-1}$ mol^{-1} k^{-1} (the universal gas constant) and ΔG = binding affinity (28). The 2D and 3D dock poses of the complexes were viewed with the Biovia Discovery Studio visualizer software (version 16.1.0). The docking targets (HMA2 and PDE5) were further subjected to MDS using the Webnm online server (http://apps.cbu.uib.no/webnma3) (29) to identify cluster and residue displacements with their structures.

Table 1. List of Ligands and their PubChem IDs.

No.	Ligand	PubChem ID
1	Sildenafil Citrate	135398744
2	Pyrogallol	1057
3	Ethyl D-glucopyranoside	11127487
4	Ethyl 2-cyano-3-methylcrotonate	136573
5	Tyrosinol	151247
6	5-Hydroxymethylfurfural	237332
7	Capric acid	2969
8	3-Fluorobenzyl alcohol	68008

ADMET Predictions

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) of the top docked compounds were predicted using the pkCSM online server (https://biosig.lab.uq.edu.au/pkcsm) (30) to further ascertain their pharmacological properties.

Statistical Analysis

Data obtained in the present study were expressed as mean ± standard error of triplicate determinations' mean (± SEM) evaluated with Statistical Package for the Social Sciences (SPSS) version 22 Software.

Results

The phytochemicals identified and quantified in ESBE of GE are presented in **Table 2**. Flavonoids were present in the highest concentration (32.67 ± 1.45%), followed by alkaloids and saponins with concentrations of 22.33 ± 1.45%, and 20.17 ± 1.88% respectively. Glycosides were detected in the least concentration (0.55 ± 0.03%), with the absence of steroids and terpenoids.

Table 2. Phytochemical composition of ethyl acetate stembark extract of *Gardenia erubescens*.

Phytochemical	Concentration (%)
Alkaloids	22.33 ± 1.45
Saponins	20.17 ± 1.88
Steroids	-
Glycosides	0.55 ± 0.03
Terpenoids	-
Flavonoids	32.67 ± 1.45

Table 3 presents the various compounds identified ESBE of *G. erubescens* showing their retention times, peak areas, molecular weights, and formulas. The fatty acid linoleic acid had the highest (28.01%) peak, followed by palmitic acid (14.08%), and 9, 17-Octadecadienal (11%). Ethyl palmitate, pentadecanoic acid, and decanoic acid were identified with peak areas of 8.03%, 4.98%, and 4.66% respectively. Other compounds identified were 5-Hydroxymethylfurfural, ethyl stearate, palmitic acid glyceryl ester, squalene, and ethyl icosanoate.

Table 3. Bioactive compounds identified in ethyl acetate stembark extract of *Gardenia erubescens*.

No.	Name of compound	Retention time	Peak area (%)	Molecular weight	Formula
1	5-Hydroxymethylfurfural	3.459	3.70	126.11184	$C_6H_6O_3$
2	3-Fluorobenzyl alcohol	4.534	0.49	126.130383	C_7H_7FO
3	Ethyl 2-cyano-3-methyl-2-butenoate	4.981	0.50	153.18084	$C_8H_{11}NO_2$
4	1,2,3-Benzenetriol	5.742	1.74	126.11184	$C_6H_6O_3$
5	Tyrosinol	5.908	0.97	167.20772	$C_9H_{13}NO_2$
6	Ethyl a-D-glucopyranoside	6.200	0.37	208.21144	$C_8H_{16}O_6$
7	Capric acid	6.978	4.66	172.2676	$C_{10}H_{20}O_2$
8	Ethyl palmitate	7.504	8.03	284.48264	$C_{18}H_{36}O_2$
9	Palmitic acid	7.853	14.08	256.42888	$C_{16}H_{32}O_2$
10	Pentadecanoic acid	8.322	4.98	242.402	$C_{15}H_{30}O_2$
11	9,17-Octadecadienal	8.958	11.00	264.45148	$C_{18}H_{32}O$
12	Ethyl stearate	9.158	3.46	312.5364	$C_{20}H_{40}O_2$
13	Linoleic acid	9.347	28.01	280.45088	$C_{18}H_{32}O_2$
14	2-Octylcyclopropane-1-carbaldehyde	10.577	1.67	182.30608	$C_{12}H_{22}O$
15	Ethyl heptadecanoate	10.783	1.93	298.50952	$C_{19}H_{38}O_2$
16	Ethyl icosanoate	10.995	2.64	340.59016	$C_{22}H_{44}O_2$
17	Myristaldehyde	11.939	0.91	212.37572	$C_{14}H_{28}O$
18	Oleic Acid	11.561	1.28	282.46676	$C_{18}H_{34}O_2$
19	Palmitic acid glyceryl ester	12.230	3.32	330.50832	$C_{19}H_{38}O_4$
20	(Z)-Nonadec-10-enoic acid	13.077	0.94	296.49364	$C_{19}H_{36}O_2$
21	Squalene	13.856	2.76	410.727	$C_{30}H_{50}$
22	(9Z)-octadeca-9,17-dienal	13.598	1.46	264.45148	$C_{18}H_{32}O$
23	Tert-Hexadecyl mercaptan	14.531	0.85	258.50596	$C_{16}H_{34}S$
24	11-Hexadecenal	15.372	0.23	238.4136	$C_{16}H_{30}O$
25	Cis-Vaccenic acid	15.893	0.02	282.46676	$C_{18}H_{34}O_2$

The structures of the identified compounds displaying their functional groups are also shown in **Figure 1**, while the chromatogram of the GC-MS analysis is present in **Figure 2**, revealing the retention time and peak areas of the compounds. GC-MS analysis identified 25 compounds in ESBE of *G. erubescens*, highlighting the chemical diversity present in the extract. Most of the compounds identified were long-chain fatty acids and a few aromatic compounds, which isn't surprising considering the oily nature of the extract. Additionally, some of the detected compounds are known for their bioactive properties, suggesting potential pharmacological applications.

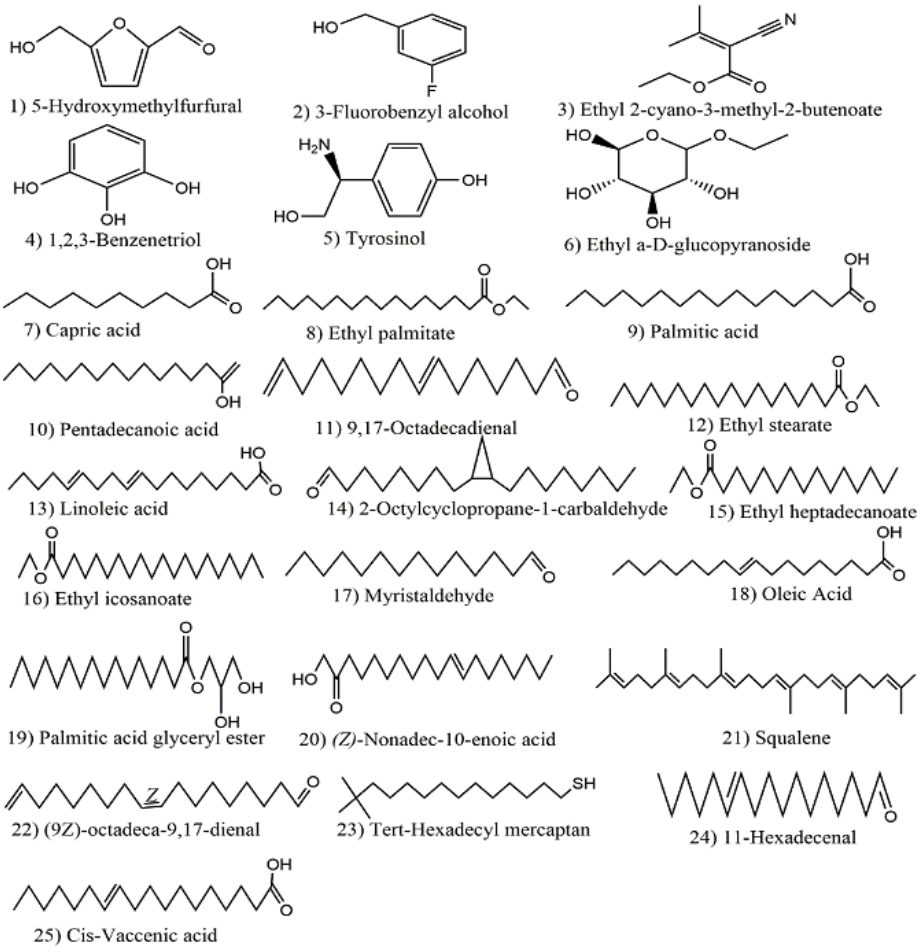

Figure 1. Structures of compounds identified in ethyl acetate stembark extract of *Gardenia erubescens*.

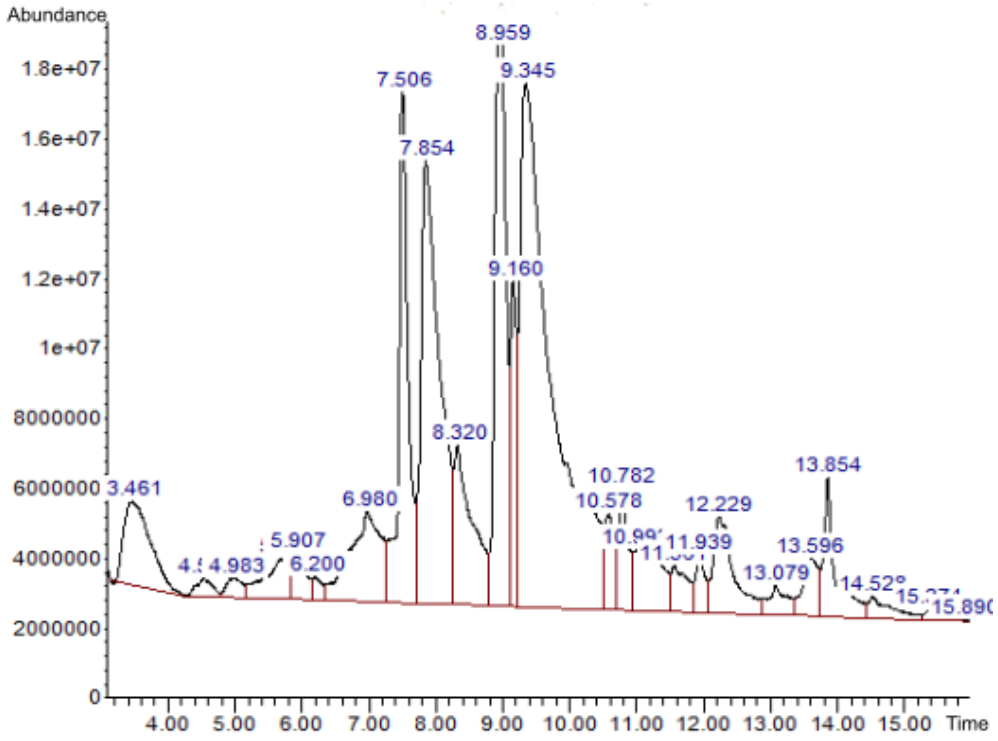

Figure 2. GC-MS chromatogram of ethyl acetate stembark extract of *Gardenia erubescens*.

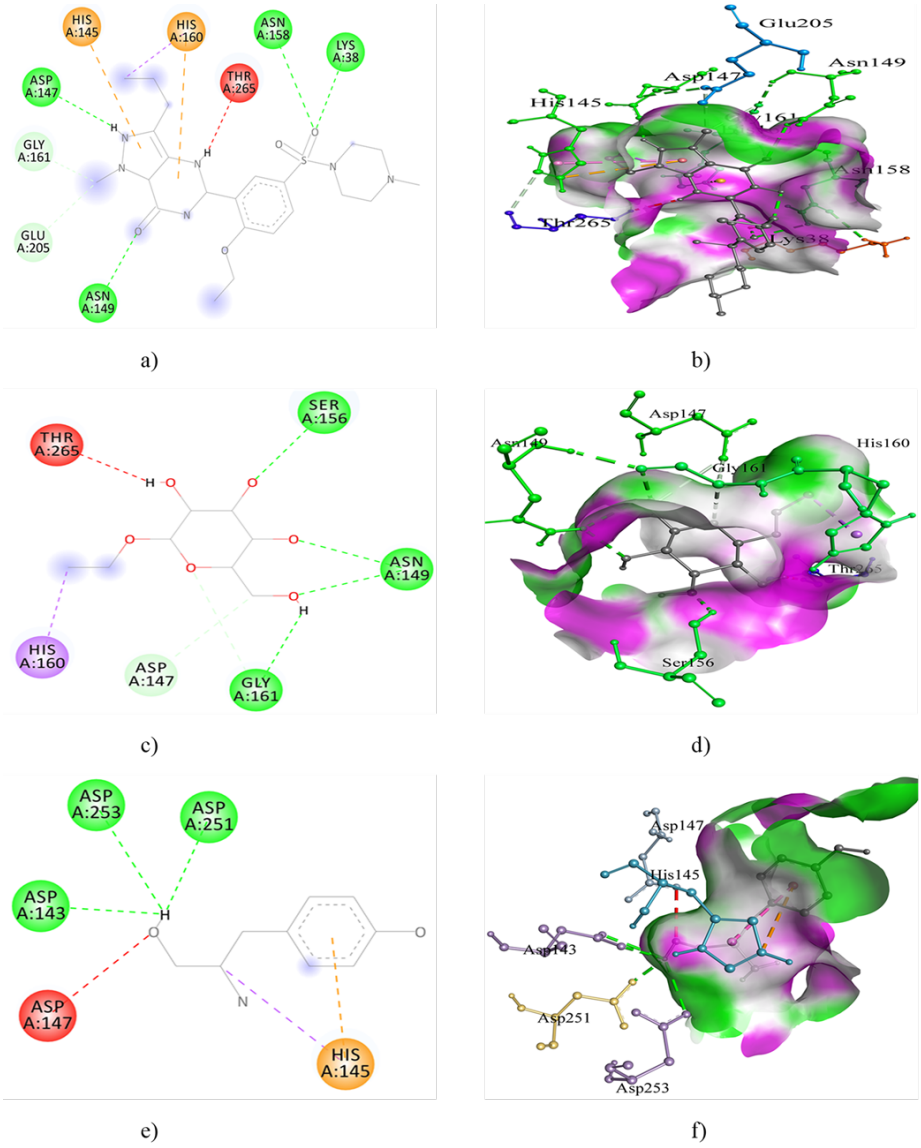

Figure 3. Docking interactions of HMA2 with sildenafil citrate; a) 2D and b) 3D, ethyl D-glucopyranoside; c) 2D and d) 3D, and Tyrosinol; e) 2D and f) 3D.

The heavy metals present in the ESBE of GE are presented in **Table 4**. Chromium (Cr) was present in the highest concentration (0.145 ppm ±0.03), followed by lead (Pb) (0.065 ppm ±0.03). Cadmium had the lowest concentration (0.001 ppm ±0.00).

Table 4. Heavy metals composition of ethyl acetate stembark extract of *Gardenia erubescens*.

Heavy metal	Concentration (ppm)
Chromium (Cr)	0.145 ±0.03
Cadmium (Cd)	0.001 ±0.00
Lead (Pb)	0.065 ±0.03

Table 5 reveals the docking interaction of the top compounds and sildenafil citrate with HMA2 depicting the BA and Ki. Although sildenafil citrate showed the least BA (-8) and Ki (1.35 µM) than the compounds, ethyl D-glucose had the least BA (-6.3) and Ki (23.82 µM) amongst the compounds next to Tyrosinol. Furthermore, **Figure 3** shows the docking interaction of sildenafil with HMA2 depicting the binding interactions. Four conventional and carbon-hydrogen bonds (HBs) were observed with additional 3 π-interactions. The binding interactions of HMA2 with ethyl D-glucopyranoside are shown in **Figure 3**. Exactly 3 conventional and 1 HBs were observed in the interaction with π-interaction with Thr265 acting as an unfavorable donor-donor. **Figure 3** depicts the binding interactions of HMA2 with IV showing the HBs and π-interactions. Asp143, 253, and 251 participated in conventional HBs while His145 in π-cation interaction with Asp147 as an acceptor-acceptor. The docking interaction of PDE5 with sildenafil citrate and the compounds is presented in **Table 5**. The least BA (-6.2) and Ki (28.21 µM) was exhibited by Tyrosinol next to Ethyl D-glucopyranoside with -6.1 and 33.40 µM respectively among the compounds, though sildenafil showed the least BA (-9.8) and Ki (0.06 µM) than the compounds.

Table 5. Docking Interactions of HM2 and PDE5 with the compounds.

Target	Ligand	BA	Ki (µM)	Interacting amino acids	Type of interactions
HM2	Sildenafil Citrate	-8	1.35	ASN A: 149, ASN A: 158, LYS A: 38, ASP A: 147, HIS A: 145, 160 THR A: 265	Conventional Hydrogen Bond Pi- Cation Unfavorable donor
	Ethyl D-glucopyranoside	-6.3	23.82	Ser A: 156, ASN A: 149, GLY A: 161, ASP A: 147, HIS A: 160, THR A: 256,	Conventional Hydrogen Bond Carbon hydrogen bond Pi-Sigma Unfavorable donor
	Tyrosinol	-5.7	65.65	ASP A: 143, 251, 253, ASP A: 147, HIS A: 145, GLN A: 817, HIS A: 613, PHE A: 820, LEU A: 765, VAL A: 782	Conventional Hydrogen Bond Unfavorable donor Pi-Cation Conventional Hydrogen Bond Pi-Cation Pi-Pi Stacked Pi-Alkyl
PDE5	Sildenafil Citrate	-9.8	0.15	GLN A: 775, 819, TYR A: 612, ALA A: 767 LEU A: 765 VAL A: 782	Conventional Hydrogen Bond Pi-Alkyl
	Tyrosinol	-6.2	28.21	HIS A: 613, 657, ASP A: 764	Conventional Hydrogen Bond Unfavorable donor
	Ethyl D-glucopyranoside	-6.1	33.40	HIS A: 685	

Moreover, the binding interaction of PDE5 with sildenafil citrate is displayed in **Figure 4**, illustrating key molecular interactions that contribute to its binding affinity. Sildenafil citrate exerted a conventional hydrogen bond (HB) interaction with Gln817, which stabilizes the binding complex. Additionally, it demonstrated multiple π-interactions, including π-cation, π-stacking, and π-alkyl interactions with residues His613, Phe820, Leu765, and Val782, respectively. These interactions enhance the molecular recognition and affinity of sildenafil citrate towards the PDE5 active site, supporting its efficacy as a PDE5 inhibitor.

Similarly, the binding interaction of PDE5 with Tyrosinol is depicted in **Figure 4**, showcasing its structural engagement within the enzyme's active pocket. Tyrosinol formed conventional HBs with Tyr612, Gln775, and Gln817, which help in stabilizing its interaction. Furthermore, it exhibited additional π-alkyl interactions with Ala767, Val782, and Leu765, reinforcing the molecular docking results. These interactions suggest that Tyrosinol may have a potential role in PDE5 inhibition, though its binding affinity would need further validation through experimental studies.

The binding interaction of PDE5 with Ethyl D-glucopyranoside is also illustrated in **Figure 4**, highlighting its hydrogen bonding and molecular interactions. Ethyl D-glucopyranoside was involved in three HBs with His613, His657, and Asp764, which contribute to its interaction with the enzyme. Additionally, it exhibited an unfavorable donor-donor interaction with His685, which might slightly affect its binding stability. Despite this unfavorable interaction, the presence of multiple hydrogen bonds suggests a moderate binding potential.

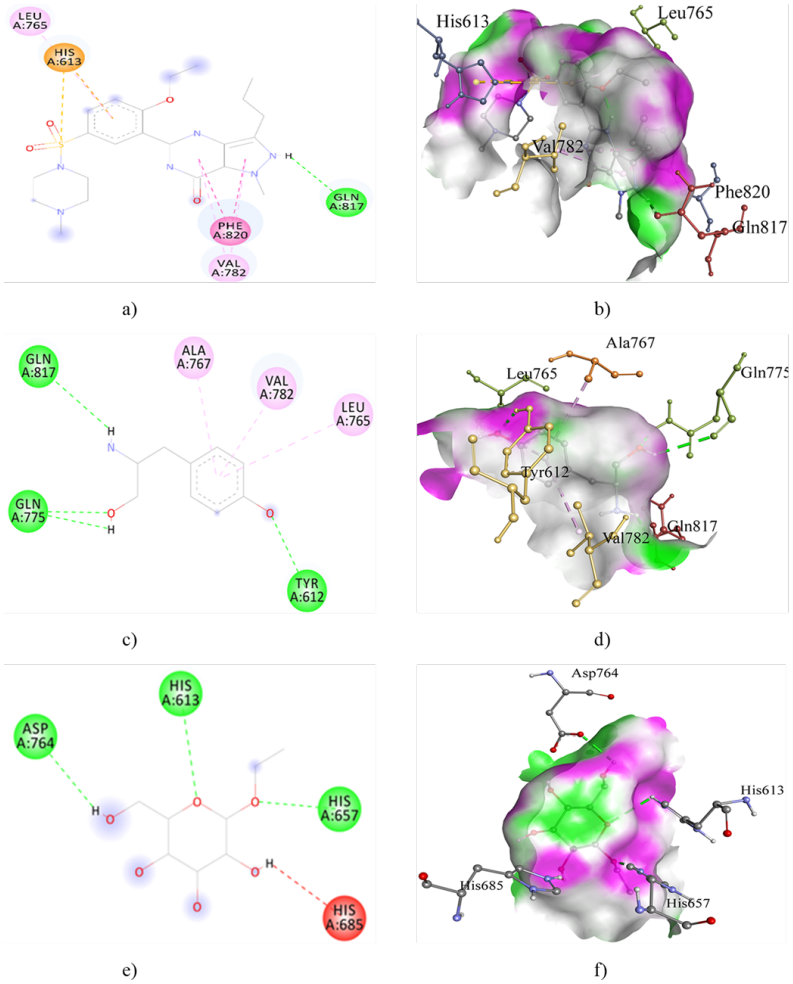

Figure 4. Docking interactions of PDE5 with sildenafil citrate; a) 2D and b) 3D, Tyrosinol; c) 2D and d) 3D, and Ethyl D-glucopyranoside; e) 2D and f) 3D.

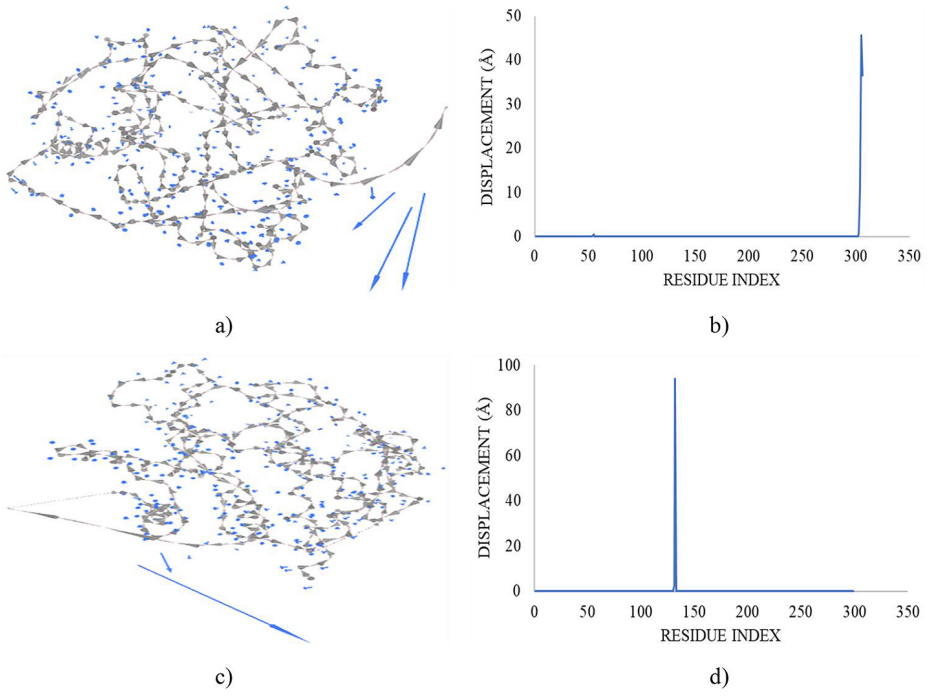

Figure 5. MDS result of docked HM2 complex depicting; a) cluster and b) residue displacements, and PDE complex depicting; c) cluster and d) residue displacements.

Table 6. ADMET predictions of the top docked compounds.

ADMET Properties		INB	II	IV
Absorption	Water solubility (log mol/L)	-3.58	-0.11	-1.17
	Human Intestinal absorption (%)	75.29	34.19	61.59
	Skin permeability (log Kp)	-2.74	-3.51	-2.52
	P-glycoprotein substrate	Yes	No	Yes
	P-glycoprotein I inhibitor	No	No	No
	P-glycoprotein II inhibitor	No	No	No
Distribution	Volume of distribution [VDss (log L/kg)]	0.95	-0.43	0.45
	Human fraction unbound	0.12	0.85	0.61
	BBB permeability (log BB)	-1.28	-1.22	-0.27
	CNS permeability (log PS)	-3.64	-4.70	-2.73
Metabolism	CYP2D6 substrate	No	No	No
	CYP3A4 substrate	No	No	No
	CYP1A2 inhibitor	No	No	No
	CYP2C19 inhibitor	No	No	No
	CYP2C9 inhibitor	No	No	No
	CYP2D6 inhibitor	No	No	No
	CYP3A4 inhibitor	Yes	No	No
Excretion	Total clearance (log ml/min/kg)	0.21	0.73	0.96
	Renal OCT2 substrate	No	No	No
Toxicity	Human max. tolerated dose (log mg/kg/day)	0.34	1.90	0.46
	hERG I inhibitor	No	No	No
	hERG II inhibitor	Yes	No	No
	LD50 [rats (mol/kg)]	2.43	1.54	2.22
	Hepatotoxicity	Yes	No	No
	Skin sensation	Yes	No	Yes

The MDS result of HMA2 is displayed in **Figure 5** depicting the residue and cluster displacements. The highest residue displacement was observed at the tail end at residues 304, 305, and 306 with displacement values of 11.23Å, 45.62Å, and 36.60Å respectively while the other residues had <1Å displacement. **Figure 5** shows the residue and cluster displacement of PDE5 during the MDS. Similar to the result observed in HMA2, the displacement here is around the mid-chain with the highest at residue 132 with a displacement of 93.98Å next to 131 with 2.73Å while the other residues had <1Å.

Table 6 shows the ADMET predictions of sildenafil and the top compounds. The least (-3.58 log mol/L) water solubility was exhibited by sildenafil (INB) while ethyl D-glucopyranoside (II) had the highest value (-0.11 log mol/L). Moreover, only Tyrosinol (IV) was predicted to be a P-glycoprotein substrate among the compounds while none were P-glycoprotein I and II inhibitors including INB. Additionally, the compounds were predicted to be neither CYP2D6 and CYP3A4 substrates nor CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 inhibitors. Additionally, INB exhibited the highest (0.95 log L/kg) steady-state volume of distribution (VDss). Furthermore, all the compounds had lower blood-brain barrier (BBB) penetration values than INB (-1.28 log BBB), though II (-1.22 log BBB) had the lowest among the compounds. Moreover, the central nervous system (CNS) permeability value of II (-4.70 log PS) was lower than all the compounds including INB (-3.64 log PS).

The highest maximum tolerated dose was exhibited by II (1.90 log mg/kg/day) higher than INB (0.34 log mg/kg/day). Moreover, the compounds were neither hERG I and II inhibitors nor hepatotoxic, however, IV was predicted to have skin sensation. INB was predicted to be a hERG II inhibitor, hepatotoxic with skin sensation. Furthermore, IV had the highest (2.22 mol/kg) LD_{50} value amongst the compounds though lower than INB (2.43 mol/kg).

Discussion

The presence of phytochemicals in an extract can be influenced by the solvent employed for the extraction attributed to the affinity of the phytochemicals for the solvent (31, 32). Thus, the presence and absence of

the phytochemicals in the present study might be influenced by the extracting solvent partly due to its polarity. The flavonoid value (32.67 ± 1.45%) reported in the present study was lower than the value (0.21 ± 0.001%) previously reported (33). The present study agrees with a previous study on the detection of alkaloids and flavonoids, though the concentrations of alkaloids (7.70 ± 0.32%) and flavonoids (12.20 ± 1.22%) were lower than the values (22.33 ± 1.45% and 32.67 ± 1.45%, respectively) reported in the present study (34). In another study on ethanol leaf extract of GE, alkaloids, saponins, and flavonoids were detected, with the absence of terpenoids and glycosides, partially agreeing with the present study (35).

Heavy metals are vital raw materials in many industries and often get released into the environment as waste in the air and water. Exposure to heavy metals leads to oxidative stress by the generation of reactive oxygen species (ROS) subsequently damaging DNA, proteins, and lipids (36). Chromium (Cr) exists in several oxidation states and causes oxidative stress in its hexavalent form (+6) which is a strong oxidizing agent leading generation of ROS such as superoxide ion, hydrogen peroxide, and hydroxyl radical, thus causing oxidative stress (37). A high concentration of cadmium (Cd) causes toxicity by binding to the protein metallothionein leading to hepatotoxicity which further circulates to the kidney causing nephrotoxicity (36). Lead (Pb) causes toxicity by the generation of ROS and depletion of antioxidants leading to oxidative stress and subsequent damage to proteins, DNA, and membranes (36, 38). The values of heavy metals concentrations reported in the present study were lower than the acceptable regulatory standards which are 1.30, 0.02, and 2 ppm for chromium, cadmium, and lead respectively (39) thus, the plant might be safe for occasional use.

L-arginine is the substrate for the synthesis of nitric oxide catalyzed by nitric oxide synthase (40). Nitric oxide mediates penile erection via the second messenger cyclic guanosine monophosphate promoting penile erection by vasodilation and relaxation (40). The arginase II enzyme catalyzes the conversion of L-arginine to L-ornithine and urea (41). Thus, inhibition of the enzyme prevents the hydrolysis of the L-arginine increasing its bioavailability for the erection process. In the present study, though sildenafil citrate exhibited superior docking than the compounds, Ethyl D-glucopyranoside and Tyrosinol binding to HMA2 might inhibit its activity contributing to the aphrodisiac effect of GE. PDE5 opposes nitric oxide activity, inhibiting the mediation of the penile erection signaling by catalyzing cyclic guanosine monophosphate in addition to decreasing the nitric oxide concentration (42). Thus, this enzyme is a target of many aphrodisiacs, lowering its activity and allowing prolonged nitric oxide effects. In our study, both capric acid and Tyrosinol interacted with this enzyme with low BA and Ki which might lead to the inhibition of its activity, though sildenafil citrate showed superior docking interaction.

The ADMET study predicts the pharmacological properties of a compound. A molecule with intestinal absorption <30% is considered poorly absorbed (30), thus, in our study, all the compounds are absorbable. A log Kp value >-2.5 is considered less skin permeant (30). In our study, all the compounds are not skin permeant. P-glycoproteins participate in the cellular xenobiotics' extrusion (30). In our study, only Tyrosinol (IV) was predicted to be a P-glycoprotein substrate while none were P-glycoprotein its inhibitors. VDss values <-0.15 and >0.45 are considered low and high respectively (30). Thus, in our study, all the compounds have low VDss. The log BBB values of >0.3 and <-1 are considered readily and poorly BBB permeable respectively (30). Thus, all the compounds are poorly distributed across the BBB in this study. Log PS >-2 and <-3 are considered CNS permeable and not permeable respectively (30). Thus, all the compounds are not CNS permeable in our study. A maximum tolerable dose <= 0.477 and > 0.477 log mg/kg/day are regarded as low and high respectively (30). Thus, only II has a high tolerance among the compounds. Moreover, the compounds were neither hERG I and II inhibitors nor hepatotoxic, however, VI and IV were predicted to have skin sensation.

Conclusion

Erectile dysfunction is a state of recurrent and persistent inability to achieve and/or maintain sufficient erection for sexual intercourse to satisfaction. Different remedies are employed including therapeutics, though improved lifestyle is recommended, however, the drugs are often with adverse effects. Thus, the prospect for alternatives such as plant sources. Conclusively, Tyrosinol and Ethyl D glucopyranoside might be responsible for the aphrodisiac effect of *G. erubescens* interacting with HM2 and PDE5. These compounds further showed promising pharmacological properties which might serve as a novel source of therapeutics against erectile dysfunction. Thus, the present study supports the folkloric aphrodisiac application of *Gardenia erubescens*, and the heavy metals level was below the acceptable regulatory level, thus, might be safe for occasional use.

Declarations

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Conflict of Interest
The authors declare no conflicting interest.

Data Availability
The unpublished data is available upon request to the corresponding author.

Ethics Statement
Not applicable.

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